CLAIMS

We claim:

1. A method for detecting the presence of an enterovirus in a clinical sample comprising the steps of:

(i) obtaining a clinical sample from a subject;

(ii) purifying RNA contained in the sample;

(iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;

(iv) contacting at least a portion of the cDNA with

(a) a composition that promotes amplification of a nucleic acid and

(b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;

(v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus amplicon is produced whose sequence comprises a nucleotide sequence of at least a portion of the VP1 gene of the enterovirus genome; and

(vi) detecting whether the amplicon is present;

wherein the presence of the amplicon indicates that an enterovirus is present in the sample.

2. The method as described in claim 1, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

3: The method as described in claim 2, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

4. The method as described in claim 3, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

5. The method as described in claim 4, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

6. The method as described in claim 2, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86.

7. The method as described in claim 6, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence

comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

8. The method as described in claim 7, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

9. The method as described in claim 1, wherein the amplification procedure comprises a polymerase chain reaction.

10. The method as described in claim 1, wherein the sample is chosen from the group consisting of whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

11. The method as described in claim 1, wherein the detection is carried out by a procedure chosen from the group consisting of gel electrophoresis and visualization of amplicons contained in a resulting gel, capillary electrophoresis and detection of the emerging amplicon, probing for the presence of the amplicon using a labeled probe, and labeling a PCR primer employed in the method and detecting the label.

12. A method for typing an enterovirus in a clinical sample comprising the steps of:

(i) obtaining a clinical sample from a subject,

(ii) purifying RNA contained in the sample,

(iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;

(iv) contacting at least a portion of the cDNA with

(a) a composition that promotes amplification of a nucleic acid and

(b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the non-polio enterovirus genome;

(v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus sample amplicon is produced whose sequence comprises a nucleotide sequence of at least a portion of the VP1 region of the enterovirus genome;

(vi) determining that the sample amplicon is present;

(vii) determining at least a partial nucleotide sequence of the sample amplicon;

(viii) providing a database consisting of prototypical nucleotide sequences, wherein each prototypical sequence is the sequence of a standard amplicon obtained from a member of a set of prototypical enterovirus serotypes by carrying out the procedure of steps (ii) through (v) on each prototypical enterovirus serotype, wherein each prototypical sequence comprises at least a portion of the sequence of the VP1 gene, and wherein the sequence of each prototypical VP1 gene is different from the sequence of every other prototypical VP1 gene in the database;

(ix) comparing the sequence of the sample amplicon with each prototypical sequence in the database; and

(x) identifying the prototypical sequence that has the highest extent of identity to the sequence of the sample amplicon to provide an identified serotype;

wherein the type of the sample is the serotype of the identified serotype.

13. The method as described in claim 12, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

14. The method as described in claim 13, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

15. The method as described in claim 14, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

16. The method as described in claim 15, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

17. The method as described in claim 13, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a

motif chosen from the group consisting of the sequences given by SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86.

18. The method as described in claim 17, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

19. The method as described in claim 18, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

20. The method as described in claim 12, wherein the sample is chosen from the group consisting of whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

21. The method as described in claim 12, wherein the amplification procedure comprises a polymerase chain reaction.

22. The method as described in claim 12, wherein an amplicon encompasses at least a portion of the nucleotide sequence for the VP1 gene of an enterovirus.

23. The method as described in claim 12, wherein the set of prototypical enterovirus serotypes comprises serotypes of coxsackie A viruses, coxsackie B viruses, echoviruses, and numbered enteroviruses.

24. The method as described in claim 23, wherein the serotypes of coxsackie A viruses (CA) comprise CA1 through CA22 and CA24.

25. The method as described in claim 23, wherein the serotypes of coxsackie B viruses (CB) comprise CB1 through CB6.

26. The method as described in claim 23, wherein the serotypes of echoviruses (E) comprise E1 through E7, E9, and E11 through E27, and E29 through E33.

27. The method as described in claim 23, wherein the serotypes of numbered enteroviruses (EV) comprise EV68 through EV71.

28. The method as described in claim 12, wherein determining at least a partial nucleotide sequence of the sample amplicon comprises a sequencing method chosen from the group consisting of a method using 2',3'-dideoxynucleotide chain terminators and a method using chemical degradation of terminally-labeled amplicons.

29. The method as described in claim 12, wherein comparing the sequence of the sample amplicon with each sequence in the database employs a sequence alignment and comparison algorithm.

30. An oligonucleotide comprising, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of a sequence given by SEQ ID NO:80, a sequence given by SEQ ID NO:81, a sequence given by SEQ ID NO:82, a sequence given by SEQ ID NO:83, a sequence given by SEQ ID NO:84, a sequence given by SEQ ID NO:85, and a sequence given by SEQ ID NO:86, or an oligonucleotide complementary to any of them.

31. The oligonucleotide described in claim 30 wherein the oligonucleotide consists of a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from the group consisting of SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or an oligonucleotide complementary to any of them.

32. An oligonucleotide whose sequence comprises, at the 3' end thereof, a sequence chosen from the group consisting of the sequence given by SEQ ID NO:3, the sequence given by SEQ ID NO:4, the sequence given by SEQ ID NO:9, the sequence given by SEQ ID NO:19, the sequence given by SEQ ID NO:20, the sequence given by SEQ ID NO:21, and the sequence given by SEQ ID NO:22, or an oligonucleotide complementary to any of them.

33. The oligonucleotide described in claim 32 whose sequence consists of a sequence chosen from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide complementary to any of them.

34. A mixture of oligonucleotides comprising at least two oligonucleotides, wherein at least one of the oligonucleotides hybridizes to a sense strand of a double stranded nucleic acid and at least one of the oligonucleotides hybridizes to an antisense strand of the nucleic acid, the nucleic acid encoding at least a portion of the VP1 gene of an enterovirus, wherein the oligonucleotides hybridize to sequences that are highly conserved among enteroviruses, and wherein the oligonucleotides, when

hybridized to the nucleic acid, direct the synthesis of an amplicon encoding at least a portion of the VP1 protein of enteroviruses when the oligonucleotides are employed in an amplification procedure using the nucleic acid.

35. The mixture of oligonucleotides as described in claim 34, wherein each oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to the nucleic acid.

36. The mixture of oligonucleotides as described in claim 34, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

37. The mixture of oligonucleotides as described in claim 34, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

38. The mixture of oligonucleotides as described in claim 37, the mixture comprising an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

39. The mixture of oligonucleotides as described in claim 38, wherein the mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

40. The mixture of oligonucleotides as described in claim 34, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from the group consisting of SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85.

41. The mixture of oligonucleotides as described in claim 40, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

42. The mixture of oligonucleotides as described in claim 41, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

43. A kit comprising a mixture of oligonucleotides, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

44. The kit as described in claim 43, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

45. A kit comprising a mixture of oligonucleotides, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

46. The kit described in claim 45 wherein the mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.